THE CONSTITUTION OF THE BASES AND COLOURING MATTERS

From the Seeds of

G A L E G A O F F I C I N A L I S.

Thesis presented for the Degree of Ph.D.

by

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July 1923.

Training and Experience.

Educated at Allan Glen's School, Glasgow, I spent the next five years (1909-14) at the Royal Technical College, Glasgow, where I passed through the prescribed Diploma Course in Chemistry, being ultimately elected to the Associateship of the College in 1913. I was appointed "Atkinson" Bursar for 1910-11-12, and "Nobel" Research Prizeman for 1913-14, and during the latter period, carried out organic research work under the supervision of Professor G.G. Henderson, F.R.S. In 1914, I obtained the Associateship of the Institute of Chemistry by examination in Organic Chemistry, and in 1918 was elected to the Fellowship.

Commissioned (Army) in October 1914, I spent the greater part of the next four and a half years in Gas Services, finally holding a position as Chemical Adviser on the Staff of the Central Laboratory, G.H.Q., France. I was demobilised in June 1919.

I then obtained an appointment as Research Chemist with the British Dyestuffs Corporation Ltd. and for the next two years was engaged on the investigation of problems in connection with dyestuffs and intermediate products. On the termination of my agreement in September 1921, I entered the Department of Medical Chemistry, Edinburgh University, in order to take up the study of Biochemistry.

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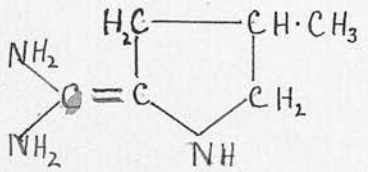
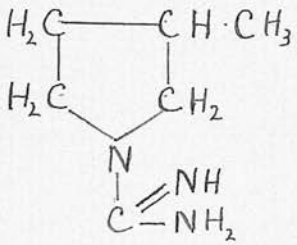
PART 1. - THE BASES.

The Constitution of Galegine.

The alkaloid, galegine, was isolated from the seeds of Galega Officinalis in 1914 by Tanret (Bull. Soc. Chim. 1914, (15), 613). By numerous analyses he showed it to have the composition $C_6H_{13}N_3$, and deduced from his results that this was composed of a combination of 3.methyl pyrrolidine and urea, with elimination of one molecule of water. He ascribed to it the formula (1), with (2) as a possible alternative:



(1)



(2)

but the evidence which he produced in support of these formulae was inconclusive, and in some respects, contradictory.

Two properties in particular were recorded, which tended to throw the gravest doubt on the correctness of either of these formulae.

The first of these properties, and one which is almost fundamental, was that, as far as Tanret was able to determine, galegine is optically inactive. Now, in a naturally-occurring base, represented by a formula which contains an asymmetric carbon atom, optical activity is certainly to be expected, and

yet/

yet Tanret's most determined efforts to resolve galegine into its active components were unsuccessful. This point alone was one which called for elucidation prior to a whole-hearted acceptance of the constitution as recorded.

With regard to the second property, Tanret obtained a dibenzoyl derivative, the existence of which is difficult to explain from the view point of either formula, since one would expect a tribenzoyl derivative from (1) and a monobenzoyl from (2).

These then, were the most outstanding arguments against either formula being a correct representation of the constitution, but there were others which were more in conformity with the second formula, than with the first. To mention one, Tanret found that in all its salts, galegine behaves as a mono-acidic base. This would mean that it is a guanidine derivative, although such naturally-occurring bodies are rare, only arginine, guanine and guanidine having hitherto been isolated. The position of the double bond in (2), however, is certainly more probable than in (1), and this suggested the possibility that galegine might give the colour reactions characteristic of guanidine bases.

Experiments on these lines were accordingly carried out, with the result that the base was found to give both Weyl's test, and the Diacetyl reaction (Harden and Norris, Centr. 1911, (2), 393), and was, indeed/

indeed, sufficiently sensitive to render colorimetric estimations possible.

Further, it was precipitated as a white silver salt, by silver nitrate in ammoniacal solution. This was, so far, evidence in favour of a guanidine nucleus.

As to the pyrrolidine nucleus, Tanret based his conclusions on the formation of an amine from the distillation of galegine at 180° C. which had the composition $C_5H_{11}N$, had a B.P. of 105°-108°C., and formed a platini-chloride which melted with decomposition at 194°-196°C. He also obtained this platini-chloride together with urea by hydrolysis with baryta.

3.Methyl pyrrolidine is recorded as having a B.P. of 103°-105° C. and forming a platini-chloride of M.P. = 194°-196° C. (Oldach, Ber., 20, 1657, 1887), but the similar properties of isomeric bodies, (piperidine, for example, boils at 106° C. and forms a platini-chloride of M.P. = 198-200° C.), suggested a reinvestigation of this amine.

Prepared by baryta hydrolysis, it was found to give a picrate of M.P. = 138° C., and an aurichloride of M.P. = 99° C., whilst the melting points recorded by Oldach for the corresponding salts of 3.methyl pyrrolidine are 105° and 170° C. respectively. That it was identical with 3.methyl pyrrolidine now appeared very unlikely, whilst

piperidine/

piperidine, whose aurichloride and picrate, prepared in a similar manner, were found to melt at 212° and 147° C. respectively, was also unlikely. (Piperidine aurichloride is recorded by Ladenburg (Ann. 247, 55) as having a M.P. of 204-206° C., and by Fenner and Tafel (Ber. 32, 3220, 1899) as melting at 215° C., whilst the picrate, as prepared by Rosenheim and Schidrowitz (J.C.S. (T) 1898, 1391) melted at 145°C.) The latter possibility was entirely eliminated when a mixture of the two picrates was found to melt between 100° and 110° C.

From a more detailed investigation of the amine, two facts emerged which changed the whole aspect of the situation.

(a) It gave Hofmann's carbylamine reaction for primary amines.

(b) It behaved as an unsaturated compound, decolourising potassium permanganate in dilute sulphuric acid solution. (Willstätter, Ber. 33, 1167, 1900).

These two facts taken in conjunction could only mean that the amine was not a ring compound, as had been believed, but an open chain compound, and from the formula ($C_5H_{11}N$), this pointed to an amine of amylene, of which there are a large number of isomers, most of them unrecorded. On this hypothesis, galegine would be an amylene derivative of guanidine, the constitution of which is quite in keeping/

keeping with Tanret's recorded results, since such a compound would probably be a mono-acidic base, would give a dibenzoyl derivative, and would not be optically active. Tanret had already noted the fact that galegine behaves as though unsaturated, and therefore the next step was obviously the reduction to a saturated compound. This was successfully carried out, galegine sulphate taking up two atoms of hydrogen to form dihydrogalegine sulphate, a saturated body which, on distillation with lime, yielded a primary amine identical with Isoamylamine.

Since dihydrogalegine gave colour reactions similar to galegine, it followed that it was probably an isoamyl guanidine. This view was established, and the position of the double bond in galegine determined, by the oxidation of galegine sulphate with barium permanganate, when three atoms of oxygen were taken up, and the products of the reaction identified as Glycocyamine and Acetone. The constitution was finally confirmed by the synthesis of dihydrogalegine from isoamylamine and cyanamide.

During these investigations, a new saturated compound was obtained from galegine sulphate by boiling with dilute sulphuric acid. On analysis, it was found that in all probability one molecule of water had been taken up to form a hydroxy derivative of dihydrogalegine sulphate $(C_6H_{15}N_3O)_2 \cdot H_2SO_4 \cdot H_2O$. This could also be regarded as a hydroxy isoamyl guanidine.

EXPERIMENTAL.

1. Preparation of Galegine Sulphate.

The method of isolation employed by Tanret was followed up to a certain point, but has been considerably simplified. The material used was a thick concentrated alcoholic extract of the seeds, 100 gm. of extract being equivalent to 1 kg. of seeds. As it still contained a considerable proportion of fats, the extract was ground with sand to a stiff paste and extracted several times with cold water, the aqueous extract being decanted off each time. The pasty mass was finally pressed on a Buchner. The combined decantations and filtrate were then treated with (a) lead acetate and (b) basic lead acetate, and the lead removed from the filtrate by means of sulphuric acid.

Tanret proceeded from this point by removing acetic acid with large quantities of ether, making alkaline with baryta, and removing the sugars by addition of excess of alcohol to the concentrated liquors. He obtained the galegine sulphate on evaporation of the alcoholic filtrate, and acidification with sulphuric acid. This rather tedious and expensive process has, however, been found to be unnecessary, and the following has been substituted.

After/

After the removal of lead, hot saturated baryta solution was added until almost all the sulphuric acid had been precipitated. (A test should show only a faint cloudiness with baryta). The barium sulphate was filtered off, and the filtrate concentrated to a thin syrup on the water bath. To this, 50% sulphuric acid was added until the solution was just faintly acid to Congo paper. On standing, galegine sulphate crystallised out, was filtered off, washed with a little distilled water, and re-crystallised from hot water. The yield obtained was equal to that claimed by Tanret, viz. 5 gm. per kg. of seeds.

The Mother Liquors.

The mother liquors, after dilution and addition of nitric acid, were treated with silver nitrate and the precipitate of silver purines and silver sulphate filtered off. The filtrate was then treated with silver nitrate and cold saturated baryta, after Kossel and Kutscher's well-known method for the isolation of arginine bases. The silver salt, washed free from barium nitrate, and ground up to a fine suspension in water, was decomposed by means of sulphuretted hydrogen, and the filtrate, after removal of H_2S was acidified with sulphuric acid until acid to litmus but not to Congo paper. The solution was then evaporated to dryness under reduced pressure, when a small further amount of galegine sulphate was obtained.

An/

An Alternative Method.

An alternative method of treatment of the mother liquors which was tried, was the precipitation by means of Dragendorff's Reagent (potassium bismuth iodide). A bright red oily precipitate was obtained from which the galegine was regenerated by grinding with freshly precipitated lead hydroxide, and extracting the mass with warm water. After removal of traces of lead with sulphuretted hydrogen, the liquor was concentrated as before. A syrup was obtained, from which it was found impossible to get the galegine sulphate to crystallise. On the addition of picric acid, however, the sparingly soluble galegine picrate was precipitated immediately. As will be shown later, however, it is a matter of difficulty to regenerate galegine from this picrate, and consequently this method of isolation is not advocated.

2. COLOUR REACTIONS.

(a) Weyl's Reaction.

2 c.c. of a very dilute solution of sodium nitroprusside were made alkaline with 2 drops of 10% caustic soda, and one drop of a 1% solution of galegine sulphate added. After standing for 15 minutes, a distinct red coloration had developed. The sensitiveness was found to be about 1 in 6000.

(b) Diacetyl Reaction (Harden and Norris).

Diacetyl was prepared from methyl ethyl ketone, amyl nitrite, and HCl (Diels and Stephan, Ber. 40, 4338, 1907) and one drop, dissolved in 5 c.c. water, was made alkaline with 2 drops of 10% caustic soda. On addition of one drop of a 0.5% solution of galegine sulphate and slight warming, the characteristic pink coloration was induced. This reaction was sensitive to 1 in 10,000, which was found by comparison to be twice that of arginine nitrate.

3. HYDROLYSIS WITH BARYTA.

Tanret obtained the amine, $C_5H_{11}N$ by heating galegine sulphate with excess of cold saturated baryta in a sealed tube at 100° C. for one hour. He then shook out the amine with ether, and extracted the ethereal solution with dilute hydrochloric acid, from which he precipitated the amine as the platini-chloride.

These directions were followed, with the exception that the acid solution was evaporated to dryness, and a white hygroscopic hydrochloride obtained, with the following properties:-

It gave a distinct carbylamine reaction with alcoholic caustic potash and a drop or two of chloroform.

About 0.05 gm. were added to 1 gm. of toluene p-sulphochloride, and shaken up with 4-5 molecular quantities of 12% caustic potash. When gently warmed, this went to a clear solution. On addition of acid a white crystalline precipitate of the toluene sulphonamide was immediately formed.

These reactions clearly showed the presence of a primary amine. That it was also unsaturated was evident from its behaviour with potassium permanganate in dilute H_2SO_4 solution, which was at once decolourised/

decolourised.

The Picrate.

A moderately soluble picrate was formed on addition of picric acid to a solution of the hydrochloride. It was recrystallised from hot water in needles of M.P. = 138° C.

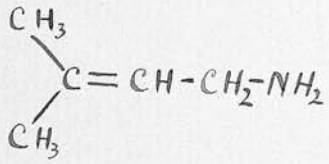
The Auri-chloride.

The auri-chloride was prepared by adding a strong aqueous solution of gold chloride to a little of the hydrochloride dissolved in a drop or two of warm water. A yellow precipitate was formed which redissolved on gently warming. On cooling, crystals separated which had a melting point of 99° C. As previously indicated, the melting points of these salts are quite unlike those given in the literature for the corresponding salts of 3.methyl pyrrolidine.

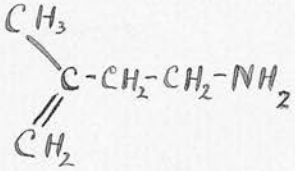
The Hydrobromide.

In a subsequent hydrolysis, hydrobromic acid wa was substituted for hydrochloric, and a hydrobromide obtained which was not at all hygroscopic. It was recrystallised from a mixture of alcohol, acetone and ether, and was obtained in colourless needles of M.P. = 200-202° C.

It will be seen subsequently that this amine is an amylene derivative not hitherto recorded, and that its formula is either -



or



4. REDUCTION.

0.88 gm. of galegine sulphate (M/400) was dissolved in 25 c.c. of water in a pressure flask, 0.02 gm. palladium chloride dissolved in a few drops of dilute hydrochloric acid added as catalyst, and hydrogen passed in under a pressure of about two atmospheres, the flask being mechanically agitated. Skita (Ber. 44, 2863, 1911) added gum arabic to keep the catalyst in solution, but this was found to be unnecessary in the present case. Absorption was complete in about three hours.

Found - Hydrogen taken up = 115 c.c. at 11° C. and 752 m.m.
= 109.4 c.c. at N.T.P.

Calc. for $(C_6H_{13}N_3)2 \cdot H_2SO_4$.

4 atoms H. (M/400) = 111.7 c.c. at N.T.P.

The catalyst, which had come out of solution, was filtered off, and the solution evaporated to a syrup under reduced pressure. This was taken up in boiling absolute alcohol, and on cooling, dihydrogalegine sulphate came out of solution as a white crystalline solid. It recrystallised from hot alcohol in colourless prisms of M.P. = 270° C. (Galegine sulphate M.P. = 227° C.)

Dihydrogalegine Sulphate.

This sulphate is sparingly soluble in alcohol and water, but more soluble than galegine sulphate. It did not decolourise potassium permanganate or bromine/

bromine water. It gave Weyl's and the Diacetyl colour reactions, but was not quite so sensitive as galegine.

Sulphate Determination.

0.2033 gm. gave 0.13362 gm. $BaSO_4$ = 27.61% H_2SO_4

Calc. for $(C_6H_{15}N_3)_2 \cdot H_2SO_4$ = 27.52% H_2SO_4

Dihydrogalegine Picrate.

On addition of picric acid to a solution of the sulphate, the picrate was thrown down immediately. It is almost insoluble in cold water, and was re-crystallised from boiling water in long narrow plates of M.P. = 172° C.

Dihydrogalegine Nitrate.

The nitrate was prepared by the addition of dilute nitric acid to a portion of the syrup from the reduction. It is sparingly soluble in dilute nitric acid, but fairly easily soluble in water and alcohol. It recrystallised from alcohol and ether in long colourless needles of M.P. = 75-76° C.

Distillation with Lime.

Thoroughly dry sulphate was finely ground with freshly ignited quick lime, in the proportions of 1 gm. sulphate to 4 gm. lime, and carefully distilled in a small flask. Ammoniacal vapours were evolved and a yellow liquid distilled over and was condensed in an ice-cold receiver. This liquid was then distilled again, when it separated into three fractions/

fractions:-

(1) A colourless liquid which came over at 89-95° C.

(2) A further small quantity of colourless liquid at 95-105° C. from which minute white crystals, similar in appearance to cholesterol, separated.

(3) A yellow viscous mass which remained in the flask, and had to be distilled in vacuo at 0 m.m. It partially sublimed and then distilled at 175° as a thick oily liquid, which solidified on cooling to a partially crystalline wax.

It was found to be insoluble in water and dilute acids, but soluble in concentrated hydrochloric acid, from which it was re-precipitated on addition of ammonia. It was also soluble in glacial acetic acid and formed a picrate from the latter solution on dilution.

The first fraction was redistilled twice, first over solid caustic potash, when it came over at 93-95°, and then over sodium and potassium when it was constant at 95-96° C. This liquid had a strong ammoniacal odour and fumed in air, in which it carbonated rapidly. It gave a white precipitate with Nessler's reagent, and the carbylamine reaction for primary amines.

The Hydrochloride.

On evaporation with excess of hydrochloric acid, a white crystalline hydrochloride was obtained, which recrystallised from acetone in long thin plates with a M.P. = 215° C.

The/

The Picrate.

On addition of excess of an ethereal solution of picric acid to the amine, a picrate was formed. This was recrystallised on standing from a solution in ether containing a trace of acetone. From a rapidly evaporating ethereal solution, it was obtained in fine needles. It melted at 130-134° C.

A sample of isoamylamine was obtained, and was found to have the same boiling point, whilst its hydrochloride and picrate crystallised in the same forms and melted at the same temperatures as those recorded. A mixture of the two hydrochlorides had a M.P. of 214° C., and the mixed picrates melted at 130-133° C. The amine obtained from dihydrogalegine was therefore ISOAMYLAMINE.

5. OXIDATION.

Kutscher (Zeit. f. phys. Chem. 32, 413, 1901) obtained guanidino-butyric acid from arginine by oxidation with barium permanganate in alkaline solution. His yield was poor, however, the greater part of the arginine going to guanidine. It was thought that better results might be obtained by carrying out the oxidation in acid solution, and this method was therefore adopted.

0.88 gm. galegine sulphate (M/400) was dissolved in 60 c.c. of 5% sulphuric acid, and 1.88 gm. barium permanganate (2 mol.), the amount calculated to yield 5 atoms of oxygen, were added in aqueous solution. The purple colour disappeared instantly, but although the mixture was heated to boiling, only 3 atoms of oxygen were taken up, and the manganese dioxide remained undissolved. The mixture of barium sulphate and manganese dioxide was filtered off whilst hot, and a thin paste of barium carbonate and water added to the filtrate until precipitation was complete. A little sulphuric acid was added, to the filtrate, until free from barium, and the clear solution evaporated to dryness under reduced pressure.

A considerable amount of white solid was left, and this was washed out with boiling alcohol, and filtered off. It recrystallised from boiling water in needle shaped plates, which on slow cooling formed/

formed characteristic sheaf-shaped clusters, which decomposed without melting about 270-280° C. This substance was very sparingly soluble in cold water, and not at all in absolute alcohol. It did not give a precipitate with barium chloride but behaved like an amino acid, in dissolving readily in dilute acids and in caustic soda. It gave a well marked Diacetyl reaction, but Weyl's reaction only very faintly. It did not give Jaffé's colour reaction with picric acid and caustic soda.

Nitrogen estimated by the micro-Kjeldahl method gave the following figures :-

(1) 6.21 mgm. gave 2.25 mgm. N_2 = 36.2%

(2) 4.5 " " 1.64 " N_2 = 36.4%

Calc. for $C_3H_7N_3O_2$ (glycocyamine) N_2 = 35.9%

The Hydrochloride.

The hydrochloride, formed by evaporating a hydrochloric acid solution of the substance, crystallised in colourless plates of M.P. = 190° C.

The Picrate.

On addition of picric acid to a solution of the substance in dilute acid, a picrate was precipitated. It was sparingly soluble in cold water, and was recrystallised from hot water, from which it separated in long needles of M.P. = 201-203° C.

Synthesis of Glycocyamine (Ramsay, Ber. 41, 4385, 1908).

A strong solution of guanidine was prepared by boiling guanidine carbonate with dilute sulphuric acid, removing the acid quantitatively with hot baryta, and concentrating the liquors. 1 gm. of chloracetic acid was then added gradually with cooling, to the concentrated liquors containing about 5 molecular proportions of guanidine. This was heated on the water bath at 60° C., when minute white crystals commenced to appear. After two hours, the reaction was complete. After cooling, the crystalline solid was filtered off, and recrystallised from 60 times its weight of water. It was found to crystallise with the same characteristic structure.

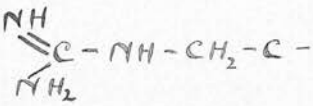
The hydrochloride and picrate were prepared, and melted at 191° and 202° C. respectively. Their crystalline forms were identical with those already prepared. A mixture of the hydrochlorides had a M.P. of 189° C., whilst the mixed picrates melted at 201° C. The oxidation product was therefore GLYCOCYAMINE.

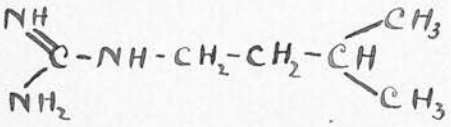
Preparation of Glycocyamidine.

As a further confirmation, a small quantity of the oxidation product was dissolved in excess concentrated hydrochloric acid, and heated in a sealed tube for two hours at a temperature of 140° C. (Barger, "The Simpler Natural Bases, p.163). The contents of the tube were then evaporated to dryness. A white substance of crystalline appearance was obtained/

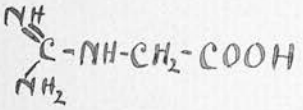
obtained, which gave with picric acid and a few drops of caustic soda, a deep blood-red colour, (Jaffé's test). The glycocyamine had therefore been converted into the anhydride, glycocyamidine.

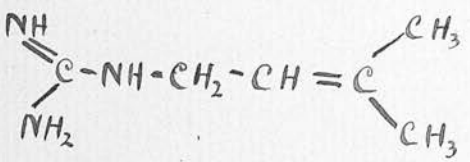
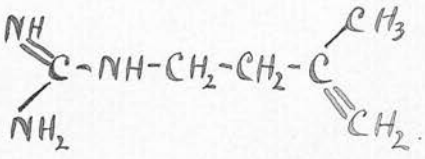
Constitution of Galegine and Dihydrogalegine.

From the results of the oxidation, it follows that both galegine and the dihydro derivative must have the grouping 

and as the latter yields isoamylamine, the complete molecule of dihydrogalegine can only be represented by 

It is therefore an isoamylguanidine.

Galegine, then, which must have been oxidised at the double bond, has yielded the compound  , which accounts for 2 atoms of oxygen. However, 3 atoms were taken up, and the remaining one must have gone to form acetone. The formula of galegine is, therefore

 or 

analogous to citronellal which Harries and Schauwecker (Ber. 34, 1498, and 2981, 1901) showed to contain the/

the $-C\overset{CH_3}{\underset{CH_2}{=}}$ group, although it yielded on oxidation, acetone and methyl adipic acid. (Tiemann and Schmidt, Ber. 29, 903, 1896, and 30, 22, 33, 1897).

To show the formation of acetone, the oxidation was repeated, using the same quantities. After addition of the permanganate, the flask was connected to a condenser, and the contents distilled. When a few c.c. of distillate had been collected, a drop of this was tested with very dilute sodium nitroprusside solution and a drop of alkali. A distinct red colouration was observed, which, on acidifying with acetic acid, was changed to purple. This indicated the presence of acetone, (Legal, Breslauer arztl. Zeit. 3 u. 4, 1883). The distillation was continued until the test was no longer given, when the total distillate was treated with a 50% acetic acid solution of nitrophenyl-hydrazine. At once a yellow precipitate was thrown down. This was collected and recrystallised from dilute alcohol. It came out of solution on cooling, in fine yellow needles which melted at 148-149° C. Yield = 0.49 gm. = 51% theory. The melting point of the nitrophenylhydrazone of acetone is given as 148-148.5° C. (Bamberger and Sternitzke, Ber. 26, 1306, 1893).

6. REGENERATION OF GALEGINE PICRATE.

Galegine picrate is difficultly soluble, requiring a large volume of boiling water to get it into solution. It is also not easily decomposed by acid, and to bring this about, it was found necessary to boil for a short time with excess of dilute sulphuric acid. On removing the picric acid and excess of sulphuric acid, and evaporating to a syrup, it was expected that galegine sulphate would crystallise out. This did not occur, but, on addition of absolute alcohol, a white solid came out of solution which differed from galegine sulphate in being extremely soluble in water. It was re-crystallised from methyl alcohol, in which it is only moderately soluble, in fern shaped crystals, not unlike ammonium sulphate in appearance, and had a melting point of 205-206° C. Galegine sulphate, it should be noted, crystallises in prisms of M.P. = 227° C., and a mixture had a melting point of less than 200° C.

From this sulphate a picrate was obtained, which was again much more soluble than the corresponding galegine salt, and in fact could only be obtained from a very concentrated solution. It recrystallised from a small quantity of hot water in rhomb-shaped crystals, of M.P. = 153-154° C., whereas galegine picrate is obtained in needles of M.P. = 180° C. This new sulphate was found to be perfectly/

perfectly stable towards potassium permanganate and bromine water. Further, it did not absorb hydrogen in presence of palladium chloride, and was therefore fully saturated. It resembled the galegine salt in giving both Weyl's and the Diacetyl colour reactions. This indicated either an intramolecular rearrangement into a ring compound, or the addition of a molecule of water at the double bond, to form a hydroxy derivative of dihydrogalegine. On analysis the following results were obtained:-

Water of Crystallisation.

A small sample of the air-dried material was heated for several hours at 120-130° C. until constant in weight.

Loss in weight (mean of 3 determinations)= 4.10%

Calc. for $(C_6H_{15}N_3O)_2 \cdot H_2SO_4 \cdot H_2O$ H_2O = 4.45%

" " $(C_6H_{13}N_3)_2 \cdot H_2SO_4 \cdot H_2O$ H_2O = 4.86%

As this appears to indicate that the sulphate contained a molecule of water of crystallisation, material dried at 120-130° C. until constant, was used for the following analyses.

Sulphate Determination.

0.2147 gm. gave 0.13002 gm. $BaSO_4$ = 25.43% H_2SO_4

Calc. for $(C_6H_{15}N_3O)_2 \cdot H_2SO_4$ = 25.26% H_2SO_4

" " $(C_6H_{13}N_3)_2 \cdot H_2SO_4$ = 27.84% H_2SO_4

Nitrogen/

Nitrogen by micro-Kjeldahl.

8.69 mgm. gave 1.89 mgm. N_2 = 21.75%

Calc. for $(C_6H_{15}N_3O)_2 \cdot H_2SO_4$ N_2 = 21.65%

" " $(C_6H_{13}N_3)_2 \cdot H_2SO_4$ N_2 = 23.86%

Combustion.

0.23 gm. gave 0.3091 gm. CO_2 = 36.65% C.

0.1696 H_2O = 8.17% H.

Calc. for $(C_6H_{15}N_3O)_2 \cdot H_2SO_4$ C = 37.11% H = 8.25%

" " $(C_6H_{13}N_3)_2 \cdot H_2SO_4$ C = 40.91% H = 7.95%

These results indicate that galegine has probably been converted into a hydroxy-dihydro galegine, and, that the transformation was due to boiling with acid, was shown by an experiment in which a small quantity of galegine sulphate was boiled with 25% sulphuric acid for five minutes. Before heating, the solution instantly decolourised potassium permanganate, but after boiling, one drop of dilute permanganate solution was sufficient to impart a permanent pink colour to the solution.

In a second experiment, 0.05 gm. of sulphate was boiled under reflux with 20 c.c. 5% sulphuric acid. After twenty minutes, 1 c.c. of solution required 3 drops of N/50 permanganate in order to retain the pink colour for one minute. At the end of thirty minutes, only 2 drops were required, and after a further fifteen minutes, 1 drop was sufficient to retain the colour.

Regeneration of Galegine Sulphate from the Picrate.

In order to obviate the boiling with sulphuric acid and consequent conversion into the hydroxy compound, the following method was adopted.

The picrate was dissolved in absolute alcohol and the solution diluted with much ether. This was shaken up with successive small quantities of 50% sulphuric acid until the acid layer was only faintly coloured. The acid extract was then shaken with small quantities of fresh ether, until colourless, when it was treated with baryta in the usual way, and the liquors evaporated under reduced pressure. By this means, some galegine sulphate was recovered, but the yield was poor (about 40% theory). This was perhaps due to the fact that although galegine picrate, like most picrates, is insoluble in ether, it is easily soluble in ether to which a very small quantity of alcohol has been added. In addition, the necessity of using an excess of acid out of all proportion to the quantity of picrate involved, renders this method very tedious.

7. Synthesis of DIHYDROGALEGINE, (ISOAMYLGUANIDINE).

Kossel (Zeit. f. phys. Chem. 68, 170, 1910) synthesised agmatine from putrescine and cyanamide by the addition of 1.1 mol. cyanamide to 1 mol. putrescine in aqueous solution, and leaving this for 17 days at summer temperature. His method has been followed in the present case.

A concentrated aqueous solution of 1 gm. cyanamide was added to 1.9 gm. isoamylamine, and the solution allowed to stand for 10 days at room temperature. It was then boiled for a short time until there was no further smell of isoamylamine, cooled and a cold saturated solution of picric acid added. A voluminous precipitate came down immediately. This was filtered off, and recrystallised from boiling water. It crystallised in long narrow plates, identical in appearance with the crystalline form of dihydrogalegine picrate. Yield = 2.1 gm. = 27% theory. After a further recrystallisation from a large quantity of boiling water, the picrate sintered at 169° C. and melted at 171° C. (Dihydrogalegine picrate M.P. = 172° C.). A mixture had a M.P. of 171-173° C.

Regeneration.

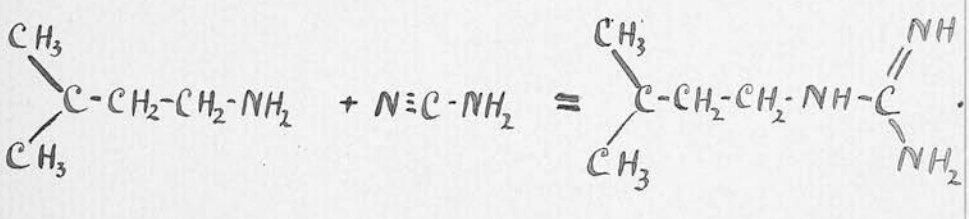
Considerable difficulty was experienced in effecting the regeneration of this picrate, in which respect it bore a strong resemblance to the galegine salt. Although boiled for about 15-20 minutes with a large excess of 10% sulphuric acid, only an

insignificant/

insignificant proportion of the picrate was decomposed, and it was found impossible to identify the product. The method finally adopted consisted in dissolving the salt in a small quantity of warm glacial acetic acid, diluting with ether, and extracting the ethereal solution many times with dilute sulphuric acid.

The acid extract was shaken up with fresh ether until free from picric acid, and the sulphuric acid quantitatively removed by means of barium carbonate, which was added in a thin paste. After filtration, the liquors were evaporated to a syrup, which was then acidified with a little dilute nitric acid. A small quantity of solid came out of solution, and was recrystallised twice from a little alcohol and a large quantity of ether. It was obtained in small colourless needles, similar in appearance to dihydrogalegine nitrate. The melting point was found to be 75-76° C. which is the same as that of the dihydro salt, and a mixture of these two substances melted at 74-76° C.

The interaction of isoamylamine and cyanamide has therefore resulted in the formation of dihydrogalegine or isoamylguanidine, and can be represented by the equation -



The yield is low, but would probably have been higher had the substances been left in contact for a longer/

longer period, as there was still a considerable amount of the amine present at the end of ten days.

Judging by the low melting point of the nitrate, it is probable that this salt is hydrated, but although dried over sulphuric acid in a vacuum for several days, there was no change in the melting point. Owing to the very poor yield obtained by the decomposition of the picrate, it was not possible to determine this point definitely.

PART II. THE COLOURING MATTERS.

I. Isolation and Identification of the Yellow Colouring Matter.

During a preliminary investigation of the alcoholic extract of Galega seeds, the presence was detected of a yellow colouring matter which gave an olive green colouration with ferric chloride, dissolved in alkali to a deep yellow solution, and was precipitated by lead acetate solution. As voluminous yellow precipitates were thrown down by lead acetate and basic lead acetate in the preparation of galegine, these were tested. The green colouration with ferric chloride was given by the former, but not by the latter, indicating that the colouring matter had been completely precipitated by the normal lead acetate. This precipitate was therefore investigated.

Experimental.

The precipitate from 600 gm. of extract (equivalent to 6 kg. seeds) was air-dried and finely ground. It was then extracted several times with boiling dilute acetic acid, in which it was almost completely soluble, and the acid solution decomposed with sulphuretted hydrogen for 24 hours. The lead sulphide was filtered off, and the filtrate concentrated under reduced pressure to small bulk. On allowing/

allowing to cool, a yellow solid came out of solution, and was collected and dried. The yield was about 2 gm. The mother liquors were evaporated in vacuo to a brown tarry mass, which was taken up in boiling alcohol and allowed to cool. No further quantity of this yellow solid could be obtained, however, the tarry material preventing any separation.

Properties of the yellow solid.

This material was quite insoluble in water, and very slightly in alcohol, but was soluble in hot aqueous alcohol. It was recrystallised from this medium, and was obtained in aggregations of small needles. It gave a deep olive-green colouration with ferric chloride, and a yellow precipitate with lead acetate, soluble in hot dilute acetic acid. It reduced Fehling's solution and ammoniacal silver nitrate. It was easily soluble in dilute caustic soda, giving a deep yellow solution. On boiling a small portion with dilute sulphuric acid, cooling and filtering, a colourless filtrate was obtained which gave Molisch's reaction. This indicated the possibility of the substance being a glucoside, and that it was easily hydrolysed.

Hydrolysis.

A.G. Perkin has shown (J.C.S. (T), 1909, 1855 and 2182) that some glucosides can be hydrolysed quantitatively by boiling with very dilute sulphuric acid./

acid. Accordingly, 0.2904 gm. (dried at 120° C.) were boiled under reflux for 3 hours with 20 c.c. 3% sulphuric acid. This was cooled, filtered, and the precipitate dried. Yield = 0.1808 gm. = 62.3%

The Sugar.

The filtrate, after removal of sulphuric acid with barium carbonate, was evaporated to dryness under reduced pressure. The residue was taken up in a little water, a small quantity of phenylhydrazine hydrochloride and sodium acetate in a little water added, and the whole heated on the water bath for one hour. Dark yellow crystals separated, which were recrystallised from alcoholic pyridine and water. They showed the characteristic structure of glucosazone, and melted at 203° C. A sample of glucosazone was prepared from pure glucose, and a mixed M.P. was found to be 204° C. The sugar was therefore GLUCOSE.

Analysis of the Glucoside.

Amethoxy determination by Perkin's method gave a negative result.

Combustion.

(1) 0.148 gm. substance gave 0.2978 gm. CO_2 = 54.93% C.
and 0.0594 gm. H_2O = 4.46% H.

(2) 0.1538 " gave 0.3104 gm. CO_2 = 55.01% C.
0.0635 gm. H_2O = 4.59% H.

Calc. for $C_{21}H_{22}O_{12}$ C = 54.07% H = 4.72%

$C_{21}H_{20}O_{11}$ C = 56.25% H = 4.46%

The/

The results obtained agreed with neither of these formulae, but were roughly mid-way between them.

A.G. Perkin (J.C.S.(T), 1902, 477), in investigating the glucoside Osyritrin or Violaquercitrin, found that it crystallised with 3 molecules of water, a half molecule of which it retained when dried at 130° C. and did not lose until heated to 160° C. The water of crystallisation was therefore determined.

0.1254 gm. glucoside were dried at 120-130° C. until constant in weight.

Loss in weight = 0.0111 gm. = 8.85%.

Calc. for $C_{21}H_{20}O_{11}\cdot 3H_2O$ Loss in weight equiv. to $2\frac{1}{2}$ H_2O = 8.96%.

0.1137 gm. of the dried substance were then heated in vacuo over phosphorus pentoxide, at the temperature of boiling tetrachlorethane. After 8 hours, the weight was constant.

Loss = 0.0022 gm. = 1.93%.

Calc. for $C_{21}H_{20}O_{11}\cdot\frac{1}{2}H_2O$ Loss equiv. to $\frac{1}{2}$ H_2O = 1.97%

The glucoside then, still retains a half molecule of water although dried at 120-130° C.

Calc. for $C_{21}H_{20}O_{11}\cdot\frac{1}{2}H_2O$	C = 55.16%	H = 4.59%
Found (1)	C = 54.93%	H = 4.46%
(2)	C.= 55.01%	H = 4.59%

The/

The equation for the hydrolysis will be represented by -

$$C_{21}H_{20}O_{11} + H_2O = C_{15}H_{10}O_6 + C_6H_{12}O_6$$

Calc. $C_{15}H_{10}O_6$ = 63.8% or, allowing for $\frac{1}{2}H_2O$ = 62.6%

Found = 62.3%.

The anhydrous glucoside melted with decomposition about 280° C.

The Split Product.

The greater part of the glucoside remaining, was hydrolysed and the split product recrystallised from aqueous alcohol in small needles. The melting point was indefinite, as it blackened between 290° and 300° C. It gave the green colour with ferric chloride and a deep yellow with dilute caustic soda.

Sublimation.

On heating in a vacuum of 0 m.m., the substance sublimed at 250-270° C. giving an almost white sublimate. It resublimed at 230-235° C.

Analysis.

After several recrystallisations, the split product, (dried at 120° C.) gave the following analysis.

(1) 0.1305 gm. gave 0.3002 gm. CO_2 = 62.73% C.
and 0.0451 gm. H_2O = 3.84% H.

(2) 0.1306 gm. gave 0.3008 gm. CO_2 = 62.81% C.
0.0463 gm. H_2O = 3.93% H.

Calc. for $C_{15}H_{10}O_6$ C = 62.94% H = 3.49%.

Acetylation.

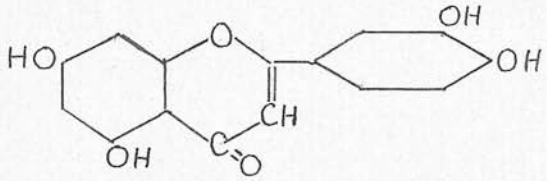
A small quantity of the split product was boiled with acetic anhydride and one drop of pyridine, as catalyst. It was cooled, poured into water and, after standing for some time, filtered. The product was recrystallised twice from a mixture of dilute acetic acid and alcohol, and separated in well defined crystals of M.P. = 215-218° C. On a further recrystallisation from absolute alcohol, it was obtained in long colourless needles which melted at 221-223° C. To make sure that acetylation was complete, this experiment was repeated using as catalyst a minute quantity of sulphuric acid. The same substance was obtained.

An analysis gave the following figures:-

0.14 gm. gave 0.3104 gm. CO_2 = 60.43% C.
and 0.0496 gm. H_2O = 3.93% H.

Calc. for $C_{15}H_{10}O_2(CH_3CO)_4$ C = 60.79% H = 3.97%

These results indicate that the split product was LUTEOLIN,



a colouring matter which has been seldom met with in nature, since Chevreul (J. Chim. méd. 6, 157) first isolated it from Weld, (Reseda luteola). It has also been obtained from Dyer's Broom, (Genista tinctoria) by A.G. Perkin and Newbury (J.C.S. (T), 1899, 830), whilst Fleischer (Ber. 32, 1184, 1899) isolated a hydroxyflavone from Digitalis purpurea,

to/

to which he gave the name "Digitoflavone", but which is undoubtedly luteolin. Although luteolin was known to be the colouring matter of Weld, it was little investigated until A.G. Perkin obtained it from that source and definitely established its constitution. (J.C.S.(T), 1896, 207 and 799; also Perkin and Horsfall, (T), 1900, 1314).

For confirmatory purposes, a sample of pure luteolin was obtained through the courtesy of Professor A.G. Perkin, F.R.S. On sublimation in vacuo, luteolin formed a similar, almost white, sublimate at 250-270° C., which resublimed at 230-235° C. The acetyl compound was prepared and compared with the acetyl compound of the split product. They both melted at the same temperature (221-223° C.) which was also the M.P. of the mixture.

The colouring matter is therefore LUTEOLIN, and as this compound has not hitherto been obtained as a glucoside, a suggested name for the latter is "GALUTEOLIN", indicating its occurrence in Galega officinalis.

The Glucoside.

In order to obtain a further quantity for more detailed examination, some Galega extract was treated as before with lead acetate. In this case, however, the precipitate was ground to a fine suspension in hot aqueous alcohol, and the lead removed with H_2S. The lead sulphide was filtered off, and the alcoholic liquors, freed from H_2S, evaporated to small bulk

under/

under reduced pressure. On cooling, an oily mass came out of solution. The clear liquid was decanted off and allowed to evaporate in vacuo at the ordinary temperature, when a yellow solid was deposited. A further quantity of this was obtained from the oily deposit by means of a series of ether extractions, the oily impurity being dissolved out. The yellow material was recrystallised many times from aqueous alcohol, but, on testing, was found to be not the glucoside, but luteolin. A small quantity of a yellow substance which gave a brown colour with ferric chloride, was also obtained from the mother liquors. On attempting to recrystallise this from aqueous alcohol, it came out in a glutinous condition, reminiscent of apiin. The quantity, however, was too small to investigate.

II. Sublimation in Vacuo as a Means of Identification of Hydroxyflavones.

Hydroxyflavones are comparatively rare substances, being usually obtained from the seeds, leaves or roots of the plant which owes its pigmentation to their presence. As they only form a very small proportion of the total organic constituents, it follows that they are seldom obtained in quantities of more than a few grams. For identification then, one does not want to utilise more than is necessary.

A melting point is generally a guide to identification, and has the advantage of using up very little of the substance, but the melting points of hydroxyflavones are invariably high, and do not afford a satisfactory means of identification. This suggested, however, the heating in vacuo as a possible alternative.

A minute quantity of the colouring matter was placed in a test tube and connected to a small motor-driven oil pump which gave a vacuum of 0 m.m. It was then placed in a metal bath which was slowly heated. The substance sublimed over a range of temperature which varied with different substances. By resublimation this range of temperature could be sufficiently narrowed to afford a means of distinguishing between the substances used. The colour of the sublimate also varied with the

different/

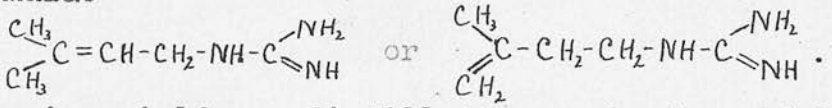
different substances. A comparison of luteolin with chrysin, apigenin and fisetin gave the following results:-

	Luteolin	Chrysin	Apigenin	Fisetin (Synthetic)
Sublimes at	250-270	220	230-290	230-330
Resublimes at	230-235	"	260-270	280-290
Colour of Sublimate	Almost white.	Faint yellow-white.	Yellow-ish White.	Brownish Yellow.

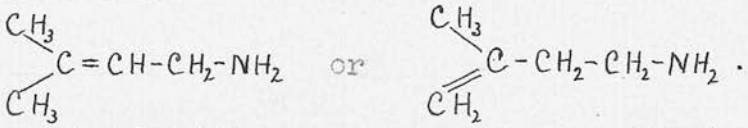
It is noteworthy that the glucoside, galuteolin similarly heated in vacuo, appears to split into its component parts, the white sublimate of luteolin appearing on the sides of the tube at the same temperature, and leaving behind the sugar as a black tarry residue.

SUMMARY.

1. Galegine has been shown to be an amylene derivative of guanidine, represented by the formulae -



2. Galegine yields on distillation, an amine which has been shown to be an amylene compound with the formulae -



The hydrobromide, picrate, and aurichloride have been described.

3. A new compound, dihydrogalegine (isoamyl-guanidine) has been prepared from galegine, and its constitution determined. The sulphate, picrate and nitrate have been described. What is probably a hydroxy amylguanidine has also been prepared, and its sulphate and picrate described.

4. Dihydrogalegine (isoamylguanidine) has been synthesised from isoamylamine and cyanamide.

5. Tanret's method for the isolation of galegine has been simplified.

6./

6. The yellow colouring matter from the seeds of Galega officinalis has been isolated and identified as Luteolin. It has been obtained in the form of a glucoside of the formula, $C_{21}H_{20}O_{11}\cdot 3H_2O$, for which the name "Galuteolin" is suggested.

7. A suggested means of identification of hydroxyflavones has been described.

In conclusion, I wish to express my deep indebtedness to Professor G. Barger for suggesting this investigation, and for his constant encouragement and advice.

My thanks are also due to the Department of Scientific and Industrial Research, without whose assistance this work could not have been undertaken.